Strong Differences in the in Vitro Cytotoxicity of Three Isomeric Dichlorobis(2-phenylazopyridine)ruthenium(II) Complexes

Aldrik H. Velders,[†] Huub Kooijman,[‡] Anthony L. Spek,[‡] Jaap G. Haasnoot,[†] Dick de Vos,[§] and Jan Reedijk*[,†]

Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands, Bijvoet Center for Biomolecular Research, Crystal and Structural Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands, and Pharmachemie BV, Swensweg 5, P.O. Box 552, 2003 RN Haarlem, The Netherlands

Received February 16, 2000

Since the discovery of the antitumor activity of cisplatin (*cis*-diamminedichloroplatinum(II), *cis*-[$PtCl_2(NH_3)_2$]),[1−3] many other metal complexes have been investigated for their possible application as antitumor drugs. One of the most promising metals appears to be ruthenium,[4,5] of which several types of complexes have shown high in vitro and in vivo antitumor activity and some compounds are in advanced stages of preclinical studies.[6−9] Although the mechanism of action of antitumor-active ruthenium compounds is not fully understood yet, it is thought that, similar to the platinum drugs,[2−5] the chloride complexes can hydrolyze[10,11] in vivo, allowing the Ru to finally bind to the nucleobases of the DNA.[12] In this communication we present the remarkably different cytotoxicity data of three isomeric dichlororuthenium(II) complexes of the type [$Ru(azpy)_2Cl_2$], in which azpy stands for the didentate ligand 2-phenylazopyridine.

The didentate azpy ligand lacks a 2-fold symmetry axis, and therefore, in theory five possible isomers of the complexes of the type [$Ru(azpy)_2Cl_2$] can be expected.[13,14] However, as yet the syntheses of only three of these isomers have been reported,[13−15] namely, α-[$Ru(azpy)_2Cl_2$], *β*-[$Ru(azpy)_2Cl_2$], and *γ*-[$Ru(azpy)_2Cl_2$], from now on referred to as α-**Cl**, *β*-**Cl**, and *γ*-**Cl**, respectively. The complexes α-**Cl** and *β*-**Cl** were suggested to have two coordinated chloride ions in a cis position and differ in the mutual orientation of the didentate ligands (Figure 1), as has been confirmed later crystallographically.[16] On the basis of spectroscopic data, the *γ*-**Cl** was originally[13,14] concluded to be the all-trans isomer in which the chlorides, the aza nitrogens, and the pyridine nitrogens are all coordinated in a trans configuration. However, the absence of a coupling between the pyridine H6 proton (of one azpy) and phenyl ring protons (of the other azpy) in a HH nuclear Overhauser effect spectroscopy (NOESY) experiment excluded the *γ*-**Cl** having the all-trans configuration in which the pyridine rings are in-plane and close to the phenyl rings of the other azpy ligand (Supporting Information). In fact, the structure of *γ*-**Cl** determined from a single-crystal X-ray diffraction study[17] shows the *γ* isomer to have only the chlorides in a trans configuration, but the pyridines as well as the aza groups are in cis geometry (Figure 2). Such a configuration has recently also been reported for a *trans*-dichlororuthenium(II) complex with the didentate ligand 1-benzyl-2-(phenylazo)imidazole[18] and indicates that the steric hindrance of the cis phenyl rings is not preventing the formation of this isomer. On the contrary, the two phenyl rings of the azpy ligands in *γ*-**Cl** are very close (the geometric centers of the phenyl rings are 3.493(8) Å apart; the angle between the ring planes is 16.9(2)°), and a strong stacking of the aromatic rings is actually likely to stabilize this configuration. A more detailed description and discussion of this complex and its spectroscopic properties will be given in a full paper on all [$Ru(azpy)_2Cl_2$] isomers.[19]

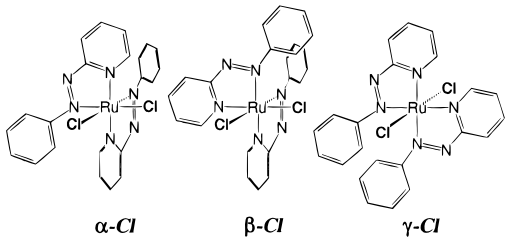

Figure 1. Structural representation of (the Δ enantiomers of) α-[$Ru(azpy)_2Cl_2$] (left), *β*-[$Ru(azpy)_2Cl_2$] (middle) and *γ*-[$Ru(azpy)_2Cl_2$] (right).

* To whom correspondence should be addressed. E-mail: reedijk@chem.leidenuniv.nl.
[†] Leiden University.
[‡] Utrecht University.
[§] Pharmachemie BV.

(1) Rosenberg, B.; Van Camp, L.; Trosko, J. E.; Mansour, V. H. *Nature* **1969**, *222*, 385.
(2) Lippert, B. *Cisplatin. Chemistry and Biochemistry of a Leading Anticancer Drug*; Verlag Helvetica Chimica Acta, Zurich; Wiley-VCH: Weinheim, 1999.
(3) Reedijk, J. *Chem. Commun.* **1996**, 801.
(4) Clarke, M. J.; Zhu, F.; Frasca, D. R. *Chem. Rev.* **1999**, *99*, 2511.
(5) Guo, Z.; Sadler, P. J. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1512.
(6) Keppler, B. K. *Metal complexes in cancer chemotherapy*; VCH: Weinheim, Germany, 1993.
(7) Depenbrock, H.; Schmelcher, S.; Peter, R.; Keppler, B. K.; Weirich, G.; Block, T.; Rastetter, J.; Hanauske, A. R. *Eur. J. Cancer* **1997**, *33*, 2404.
(8) Sava, G. *Ruthenium Compounds in Cancer Chemotherapy*; Fricher, S. P., Ed.; Chapman & Hall: London, U.K., 1994.
(9) Sava, G.; Alessio, E.; Bergamo, A.; Mestroni, G. *Metallopharm. Top. Biol. Inorg. Chem.* **1999**, *1*, 1.
(10) Velders, A. H.; Ugozzoli, F.; Biagini-Cingi, M.; Manotti-Lanfredi, A. M.; Haasnoot, J. G.; Reedijk, J. *Eur. J. Inorg. Chem.* **1999**, 213.
(11) Velders, A. H.; Pazderski, L.; Ugozzoli, F.; Biagini-Cingi, M.; Manotti-Lanfredi, A. M.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chim. Acta* **1998**, *273*, 259.
(12) Hartmann, M.; Einhauser, T. J.; Keppler, B. K. *Chem. Commun.* **1996**, 1741.
(13) Krause, R. A.; Krause, K. *Inorg. Chem.* **1980**, *19*, 2600.
(14) Goswami, S.; Chakravarty, A. R.; Chakravorty, A. *Inorg. Chem.* **1981**, *20*, 2246.
(15) Bao, T.; Krause, K.; Krause, R. A. *Inorg. Chem.* **1988**, *27*, 759.
(16) Seal, A.; Ray, S. *Acta Crystallogr.* **1984**, *C40*, 932.
(17) Crystal data for *γ*-**Cl**: $C_{22}H_{18}Cl_2N_6Ru$, M_r = 538.39, purple, needle-shaped crystal (0.05 mm × 0.05 mm × 0.40 mm), hexagonal, space group $P6_5$ with a = 22.2928(19) Å, c = 8.5121(10) Å, V = 3663.5(6) Å^3, Z = 6, D_x = 1.464 g cm^{-3}, μ(Mo Kα) = 0.9 cm^{-1}. All data, where relevant, are given without disordered solvent contribution. A total of 23 221 reflections were measured (5428 independent, R_{int} = 0.072, 1.6 < τ < 27.4, T = 150 K, Mo Kα radiation, graphite monochromator, λ = 0.710 73) on a Nonius Kappa CCD diffractometer on a rotating anode; data were corrected for absorption using platon/mulabs. The structure was solved by automated direct methods (SHELXS86). Full-matrix least-squares refinement of 281 parameters on F^2 (SHELXL-97) resulted in a final R1 value of 0.056, wR2 = 0.106, GoF = 1.19. H atoms were introduced on calculated positions. Channels parallel to the *c* axis with a volume of 388 A^3 per unit cell are filled with disordered solvent (propably diethyl ether); the associated electron density was taken into account with the platon/squeeze procedure. A total of 107 e per unit cell was found and corrected for. Final residual density was between −0.97 and 0.60 e A^{-3}.
(18) Misra, T. K.; Das, D.; Sinha, C.; Ghosh, P.; Pal, C. K. *Inorg. Chem.* **1998**, *37*, 1672.

10.1021/ic000167t CCC: $19.00
Published on Web 06/16/2000

Table 1. ID_{50} Values in ng/mL (and μmol/L in Parentheses) of **α-Cl**, ***β*-Cl**, and ***γ*-Cl**,[20] Cisplatin (CPT), and 5-FU against a Series of Tumor-Cell Lines[21]

	MCF-7		EVSA-T		WIDR		IGROV		M19		A498		H266	
α-Cl	321	(0.6)	52	(0.1)	1050	(1.9)	428	(0.8)	102	(0.2)	653	(1.2)	820	(1.5)
***β*-Cl**	2700	(4.1)	1240	(1.9)	7368	(11.2)	4801	(7.3)	1647	(2.5)	5770	(8.8)	6564	(10.0)
***γ*-Cl**	3159	(5.9)	2933	(5.4)	8948	(16.6)	6348	(11.8)	2437	(4.5)	8253	(15.3)	8016	(14.8)
5-FU	750	(5.8)	475	(3.7)	225	(1.7)	297	(2.3)	442	(3.4)	143	(1.1)	340	(2.6)
CPT	699	(2.3)	422	(1.4)	967	(3.2)	169	(0.6)	558	(1.9)	2253	(7.5)	3269	(10.9)

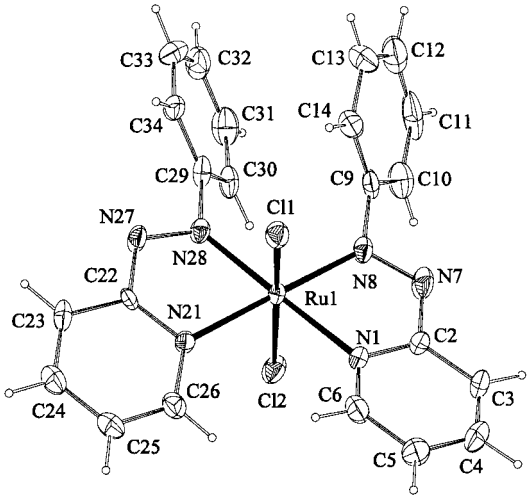

Figure 2. Displacement ellipsoid (50% probability) plot of the structure of ***γ*-Cl**. H atoms are drawn as spheres of arbitrary size. Selected bond distances (Å) and angles (deg) are the following: Ru(1)−N(1) 2.116(6), Ru(1)−N(8) 1.986(5), Ru(1)−N(21) 2.099(5), Ru(1)−N(28) 1.988(5), Ru(1)−Cl(1) 2.3768(15), and Ru(1)−Cl(2) 2.3683(16); Cl(1)−Ru(1)−Cl(2) 170.50(7), Cl(1)−Ru(1)−N(1) 88.64(14), Cl(1)−Ru(1)−N(8) 96.87(13), Cl(1)−Ru(1)−N(21) 85.71(12), Cl(1)−Ru(1)−N(28) 88.88(13), Cl(2)−Ru(1)−N(1) 85.83(13), Cl(2)−Ru(1)−N(8) 89.34(13), Cl(2)−Ru(1)−N(21) 88.15(12), Cl(2)−Ru(1)−N(28) 96.63(13), N(1)−Ru(1)−N(8) 76.4(2), N(1)−Ru(1)−N(21) 104.1(2), N(1)−Ru(1)−N(28) 177.52(19), N(8)−Ru(1)−N(21) 177.40(17), N(8)−Ru(1)−N(28) 103.8(2), N(21)−Ru(1)−N(28) 75.80(19).

In Table 1 the cytotoxicity data toward a series of human tumor-cell lines of the three above-mentioned isomers **α-Cl**, ***β*-Cl**, and ***γ*-Cl**[20] are listed and compared with the data of the well-known antitumor compounds cisplatin (CPT) and 5-fluorouracil (5-FU).[21] From the three ruthenium azpy complexes the ***β*-Cl** and ***γ*-Cl** show low to moderate cytotoxicity, while the **α-Cl** isomer is roughly a factor 10 more cytotoxic in most of the used cell lines. The cytotoxicity of **α-Cl** in fact is comparable with that of cisplatin and 5-FU, being even significantly more active than CPT and 5-FU in the fast growing cell lines: MCF-7, M19, and EVSA-T. The high cytotoxicity of the **α-Cl** is unlikely to be caused by the heterocyclic ligand because the free azpy ligand shows low cytotoxicity.

One of the main structure−activity relationships for antitumor-active platinum complexes is the presence of two cis-coordinated leaving groups, e.g., chloride ions.[2,3] If the coordination of ruthenium complexes to biologically relevant ligands such as DNA bases is important for its biological activity, the difference in cytotoxicity between the α and γ isomer might indicate that cis bifunctional coordination to the ruthenium is crucial for activity. We have recently started to study the binding of DNA (model) bases to the $[Ru(azpy)_2]$ complexes[22] and compared the results with the data of the structually relatively similar complex *cis*-$[Ru(bpy)_2Cl_2]$.[23−27] The accessibility of the two coordination sites for biological nitrogen bases, i.e., the chloride ions, appears to be different in the *cis*-dichloro complexes. The coordination of guanine derivatives to the α isomer[22] is sterically less hindered than is the case for the *cis*-$[Ru(bpy)_2Cl_2]$ complex.[24] Also the bifunctional coordination of the purine model base 1-methylbenzimidazole to the α isomer[19] is sterically less hindered than is the case for the β isomer[28] and the *cis*-$[Ru(bpy)_2Cl_2]$ complex.[27] These differences in accessibility for coordination of nitrogen heterocycles do correspond well with the higher cytotoxicity found for the **α-Cl** and do suggest that the coordinative binding to biologically relevant ligands such as the DNA purines might play a role in causing the biological activity of antitumor-active ruthenium complexes.

In conclusion, we have found that the *cis*-dichlororuthenium(II) complex α-$[Ru(azpy)_2Cl_2]$ shows remarkably high cytotoxicity against a series of tumor-cell lines, which is in contrast to the low cytotoxicity of its isomeric complexes *β*-$[Ru(azpy)_2Cl_2]$ and *γ*$[Ru(azpy)_2Cl_2]$ and the structurally related complex *cis*-$[Ru(bpy)_2Cl_2]$. Therefore, in the search for a structure−activity relationship of antitumor-active ruthenium complexes, the investigation of complexes of the type *cis*-$[Ru(LL')_2Cl_2]$ (with LL′ being a (asymmetrical) heterocyclic didentate ligand) seems very useful and at present indicates steric hindrance of the LL′ ligand toward the free coordination sites to be a crucial factor. Further studies will deal with this latter aspect and will be reported in a full paper.

Acknowledgment. The authors thank Johnson Matthey Chemicals for the generous loan of $RuCl_3 \cdot 3H_2O$. This research is sponsored by the Council for Chemical Sciences of The Netherlands Organization for Scientific Research (NWO). Also, support by COST Action D8/0017/97 (the chemistry of metals in medicine) is kindly acknowledged.

Supporting Information Available: Experimental data of the biological testing, HH NOESY spectrum of ***γ*-Cl**, and crystallographic data of ***γ*-Cl**. This material is available free of charge via the Internet at http://pubs.acs.org.

IC000167T

(19) Velders, A. H. Manuscript in preparation.

(20) The ligand 2-phenylazopyridine and the ruthenium complexes α-$[Ru(azpy)_2Cl_2]$, *β*-$[Ru(azpy)_2Cl_2] \cdot CHCl_3$, and *γ*-$[Ru(azpy)_2Cl_2]$ were prepared according to literature procedures.[13,14] The purity of the compounds was checked with elemental analyses and ^1H NMR.

(21) The cytotoxicity of (enantiomeric mixtures of) the $[Ru(azpy)_2Cl_2]$ complexes was tested in vitro applying seven well-characterized human tumor-cell lines (MCF-7 (breast cancer), EVSA-T (breast cancer), WIDR (colon cancer), IGROV (ovarian cancer), M19 MEL (melanoma), A498 (renal cancer), and H226 (nonsmall cell lung cancer)) using the microculture sulforhodamine B test (SRB) for the estimation of the cell viability (available in Supporting Information). On suggestion of a referee, the cytotoxicity values are also given in molar concentration, which is more appropriate for a comparison of compounds with different molecular weights.

(22) Hotze, A. C. G.; Velders, A. H.; Ugozzoli, F.; Biagini-Cingi, M.; Manotti-Lanfredi, A. M.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chem*., in press.
(23) Nováková, O.; Kaspárková, J.; Vrána, O.; van Vliet, P. M.; Reedijk, J.; Brabec, V. *Biochemistry* **1995**, *34*, 12369.
(24) Van Vliet, P. M.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chem.* **1994**, *33*, 1934.
(25) Grover, N.; Gupta, N.; Thorp, H. H. *J. Am. Chem. Soc.* **1992**, *114*, 3390.
(26) Grover, N.; Welch, T. W.; Fairley, T. A.; Cory, M.; Thorp, H. H. *Inorg. Chem.* **1994**, *33*, 3544.
(27) Velders, A. H.; Hotze, A. C. G.; Haasnoot, J. G.; Reedijk, J. *Inorg. Chem.* **1999**, *38*, 2762.
(28) Quiroga, A. G. Manuscript in preparation.